Hemoglobin Status Is Positively Associated with Neurodevelopment in Lead-Exposed Preschool Children from Montevideo, Uruguay

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Abstract-**Micronutrient deficiencies and environmental toxicants have been shown to contribute to children's neuro-developmental deficits. Despite intense research on anemia and lead exposure, we know little about how these two factors together affect children's development. The objective of this study was to test the association of hemoglobin (Hb) and blood lead (BLL) concentrations with measures of development in preschool children (n=69, ages 14.4 – 45.6 mo) from Montevideo, Uruguay. Children were assessed with Bayley Scales of Infant Development III (Bayley); maternal IQ, depressive symptoms and parenting stress were assessed as contextual factors. The mean BLL was 6.0 ± 3.0 μg/dL and 11.5% of children had levels ≥ 10μg/dL. The mean Hb was 12.3 ± 1.7 g/dL and 18.8% of children experienced anemia (Hb <11 g/dL). Mean composite Bayley cognitive, language and motor scores were: 90.3 ± 12.4, 90.1 ± 17.4 and 96.4 ± 14.6 points, respectively. In unadjusted regressions, higher Hb was associated with higher cognitive, language and fine motor scores (point estimates: 0.4 – 0.6, p<0.05). BLL was not associated with scores. Covariate adjustment slightly attenuated estimates for Hb (p<0.1). There was an Hb-BLL interaction on fine motor scores (p<0.15): Hb was positively associated with performance but only for children with BLL <5 μg/dL. We found an association between Hb and Bayley scores, and a suggestion of an interaction between Hb and BLL. But based on the obtained data we were unable to conclude whether children with anemia are more susceptible to the toxic effects of lead.**

Keywords-*Lead; Hemoglobin; Preschool; Cognition; Uruguay*

I. INTRODUCTION

Neuro-developmental deficits in children produce a substantial negative impact on societal and economic growth. Micronutrient deficiencies and environmental toxicants, both prevalent in young children, have been implicated as contributing to these deficits. It is estimated that 1.62 billion people worldwide have anemia, most likely due to iron deficiency [1], with the majority (~68%) living in medium and low-income countries [2]. Although it is unclear how many children globally are exposed to lead, the number appears to be high [see [3] and [4], [5] as examples]. More prevalent and severe exposures seem to occur in low- to middle-income countries, where mining, smelting, battery recycling [4] and electronic waste [6] are important sources. Beyond the potential geographic overlap in anemia and lead exposure, there is a clear association between anemia and elevated blood lead levels (BLLs) in children [7]-[10].

Given that both anemia and lead exposure occur disproportionately in disadvantaged populations, there is significant potential for millions of children to be co-exposed to these insults during the highly vulnerable periods [11] of intensive brain development, and to experience neuro-cognitive deficits that are more pronounced than in children exposed to either condition alone. In fact, studies on the separate effects of anemia due to iron deficiency (IDA) and elevated BLLs on child development suggest an enormous potential for synergisms in insults. Lead exposure in children results in deficits in IQ [12], [13], attention, visual spatial abilities [14], and executive functions [15], whereas children with IDA show altered mental, motor, and affective development [16], [17]. There are also important similarities in the effects of IDA and lead on neural processes that lend credence to the idea that when these insults occur together they could result in synergistic effects [18]. Surprisingly, despite years of intense research on anemia and lead exposure, we do not know whether lead exposure in combination with anemia has a more profound effect on the cognition or behavior of children than either insult alone. Only five studies thus far have specifically examined the statistical interactions between iron status and BLLs: two reported some effect modifications [19, 20] but three did not [21]-[23]. Others did not examine interactive effects but found that BLLs and iron status independently predicted children's cognition or behavior [24]-[30].

Here we report a study of the association between hemoglobin levels, BLLs and neuro-cognitive development of preschool children from Montevideo, Uruguay. Both anemia and lead exposure are documented problems in these children [31], [32].

II. METHODS

The study was conducted at the Parish Center of Our Lady of Fátima church, affiliated with a local university and located in the neighborhood of Cerro, Montevideo, Uruguay. The study setting and participant recruitment have been described previously. Briefly, children were recruited from among the participants of a screening study of blood lead levels and anemia, and from five preschools sponsored by the Uruguayan Institute for Children and Adolescents and located in the Cerro neighborhood of Montevideo. The previously screened families received a letter inviting them back for the present study, after which three separate contact attempts were made by telephone. For recruitment from the preschools, directors gave permission to hold informational meetings for parents to describe the study purpose, benefits and risks. In-

terested parents provided their contact information and were later contacted to set-up study appointments at the Parish Center. Thirty-six previously screened children (out of 244) returned for the current study and 88 children were newly recruited from the preschools, for a total of 124 children. The previously screened children who returned for the study did not differ on any exposure or demographic characteristics from those who did not to participate (data not shown). Based on an *a priori* decision, if parents brought siblings for the evaluation, only the youngest eligible child was included in the study. The 109 participants ranged in ages between 13 and 55 months.

Ethics Committee at the participating institutions approved the study. Written informed consent was obtained from the parents by the coordinating pediatrician or psychologist when potential participants arrived for the first study visit.

A. Procedure

Children and parents were invited to attend two separate evaluation sessions at the Parish Center: one to collect biological samples, anthropometric measurements, and demographic questionnaires, the other to assess maternal IQ, depression, and stress as well as child cognition. The visits lasted 1.5-2 hours and were scheduled approximately one week apart. At the end of the study, children received an age-appropriate toy for their participation. Parents were provided compensation for their transportation to the Parish Center by bus.

B. Hemoglobin and Blood Lead Determination

A phlebotomy nurse collected a non-fasting venous blood sample. A drop of blood was taken immediately after the blood draw for the analysis of hemoglobin via a portable instrument (Hemo Cue, Lake Forest, CA), which was calibrated daily using standard controls. Whole blood was transported on ice to the Department of Toxicology and Environmental Hygiene, Faculty of Chemistry, University of the Republic, where it was stored at -20°C until analysis. A venous blood draw could not be completed for 10 children due to difficulties with finding a vein or distress. Instead, a few drops of capillary blood were used to determine hemoglobin level, after wiping the puncture site with alcohol and removing the first drop of blood. Blood lead analyses were performed as described previously at the Laboratory —"EQUIMTOX" (Specialized Center for Chemical Toxicology), Department of Toxicology and Environmental Hygiene, University of the Republic of Uruguay, using atomic absorption spectrometry. The laboratory participates in the Inter-laboratory Program for Quality Control for Lead in Blood, Spain (PICC-PbS, 2001) and the U.S. Centers for Disease Control and Prevention's Lead and Multi-Element Proficiency Program.

C. Maternal Assessments

1) Demographic Questionnaire:

Mothers responded to questions about the household and family characteristics, including parental age, schooling and occupation, family size, size and characteristics of the dwelling, and household possessions.

2) Home Observation for Measurement of the Environment (HOME) Inventory:

An abbreviated version of the HOME was used to assess children's home environments (separate instruments for children <3 years and 3-6 years) because home visits were not logistically possible. This abbreviated version of the original HOME Inventory [33] was adapted from the instruments used in U.S. Bureau of Labor and Statistics National Longitudinal Surveys (NLSY79 Children and Young Adults) [34]. The questionnaire was translated into Spanish by the study team and confirmed by a translator not affiliated with the study. The questions assessed the number of books and toys the child has, how often parents read to the child, how many times per week the child leaves the house to go to the supermarket and other outings, how often the child spends time/eats meals with both biological parents.

3) Wechsler Adult Intelligence Scale III (WAIS III):

Five sub-tests of the WAIS III (TEA Ediciones, S.A., Madrid, Spain) were used to assess maternal IQ (similarities, arithmetic, vocabulary, block design, and object assembly), and the scores were used to calculate an estimated IQ based on previously published methodology [35]. Each test was scored by two researchers to minimize errors, with differences resolved in discussion with the test administrator and the coordinating psychologist.

4) Beck Depression Inventory II (BDI II):

The presence of maternal depressive symptoms was assessed using the Argentine adaptation of the BDI II (Ediciones Paidós, Buenos Aires, Argentina). The women's answers to the 21 items on the inventory were summed to obtain a total BDI score, to determine mild (BDI scores 14-19 points), moderate (20-28 points) or severe (29-63 points) depressive symptoms.

5) Parenting Stress Index, 3rd Edition (PSI):

Feelings of stress due to parenting a young child were assessed using the PSI (Psychological Assessment Resources, Inc; Lutz, FL). The 36-item index yields the Total Stress (TS) score and three sub-scale scores: Parental Distress, Parent-Child Dysfunctional Interaction, and Difficult Child. TS score indicates the overall level of stress an individual experiences in the parenting role (exclusive of other life stresses or events). The points obtained on the questionnaire were converted to T-scores; T-scores >90 indicate clinically significant level of stress.

All cognitive/psychological assessments were individually administered to the mothers by trained psychologists or advanced psychology students who were trained extensively by the coordinating psychologist before the study began.

D. Child Assessment

Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley, Harcourt Assessment, San Antonio, TX) was used to assess the development of any participating children who were less than 42 months of age. The Bayley III is a validated instrument that assesses several domains of child development. In this study, cognitive, language (including receptive and expressive communication), and motor (including fine and gross motor) abilities were evaluated.

The test was administered in Spanish, with the translation kindly provided by Lourdes Schnaas from the National Institute of Perinatology. The assessment was conducted by advanced psychology students from a local university. These testers were experienced in cognitive test administration to children prior to working with the Bayley III and underwent an extensive training session with children of similar ages who were not part of the study. Select sessions were videotaped and analyzed for training purposes.

Testing took place in an isolated room, with only the tester and child (alone or with parent) present. The child was either seated on the caregiver's lap (with the caregiver asked not to aid or participate in the testing) or sat independently in a chair without the caregiver present. These arrangements depended mainly on the child's age and comfort level with the parent's absence. The scales of interest were administered in one session unless the child became visibly fatigued, fussy or uncooperative, in which case the testing was rescheduled for another day.

E. Statistical Analysis

1) Variable Creation:

Based on our previous research, maternal employment was dichotomized as employed outside or unemployed. Anemia was defined as hemoglobin <11.0 g/dL. A continuous variable for SES (range 0-12 points) was created by summing the total number of affirmative responses indicating ownership of household items/appliances: television set, refrigerator, DVD player, washer, computer, house phone, video games, radio, car, sound equipment, cellular phone, video player. Occupant density was calculated as the number of persons living in the house divided by the number of rooms, indicated crowding in the home, and was included as a continuous variable in the analyses.

2) Dependent Variables:

Children's scores on the five scales (cognitive, receptive language, expressive language, fine motor, and gross motor) were treated as dependent variables in regression models. The main analysis was conducted using age-scaled scores but because the Bayley III was not standardized for the Uruguayan population, all analyses were repeated with raw scores.

3) Covariate Selection:

Potential covariates were based on previous studies of lead exposure and child neurobehavioral outcomes, and included child's age (months) and sex, whether the child lived with one or both parents, maternal age (years), years of schooling, maternal IQ score, BDI score, PSI TS score, employment status, the parents' marital status, monthly income (fixed or variable), SES score, occupant density, HOME Inventory score, and tester. The final set of covariates was chosen based on the p-values (<0.1) obtained in bivariate regressions modeling the five outcomes (cognitive, receptive and expressive language, fine and gross motor scales) as dependent variables and the potential covariates as independent variables. The final set of covariates was selected if associated with three of the five scale scores, and included, for scaled scores: child's age in months, maternal IQ, BDI score, PSI score, housing occupant density, HOME Inventory score, and tester; for raw scale scores: child's age in months, BDI score, mother's employment status, HOME Inventory score, and tester.

4) Identification of Analyzable Sample:

Children were excluded from analysis if they did not have complete information on the outcomes or potential covariates, as follows: 23 children were excluded because they did not have information on maternal IQ, 2 for being lack of PSI scores, 1 for lack of occupant density information, and additional 14 for not having Bayley scores, which gives an analyzable sample of 69.

5) Regression Modeling:

To understand the independent and interactive effects of

hemoglobin and blood lead concentrations on children's scores, we created ordinary least squares (OLS) regression models for each Bayley scale. In the first type of model (Model 1), hemoglobin was entered as the sole predictor. In Model 2, BLL was entered as the sole predictor. In Model 3, the main effects of hemoglobin and BLL were tested together, and in Model 4, the interaction between hemoglobin and BLL was tested. To create the interaction term between two continuous variables, hemoglobin and BLL were centered at each variable's mean. If the interaction term was not significant ($p<0.15$), the main effects model was reported. If the interaction was significant, it was further explored in stratified analysis, modeling BLLs as the independent variable for children who did and did not have anemia, and modeling hemoglobin for children with and without elevated BLL ($\geq 5\mu g/dL$). To account for other variables that may influence children's Bayley performance, Models 1-4 were repeated to include the final set of covariates (see above). In addition, anemia and elevated BLLs ($\geq 5\mu g/dL$) and their interaction terms were modeled as predictors. All analyses were performed with STATA 10 (STATA Corp., College Station, TX).

III. RESULTS

A. Sample Characteristics

Children who were excluded from analysis were older (33.7 ± 11.5 vs. 29.9 ± 45.6 mo, p=0.049); this is because many were not evaluated with the Bayley, which was an exclusion criterion. They also had slightly lower HOME Inventory scores (8.0 ± 3.1 vs. 9.1 ± 2.0 points, p=0.03). No other differences were noted. The participating children (ages 14.4 – 45.6 mo) experienced low-level exposure to lead, with 52.5% having BLLs ≥ 5 µg/dL and 11.5% having BLLs ≥ 10 µg/dL. The mean hemoglobin concentration was within normal range for this age group (Table 1), but 18.8% of children experienced mild anemia (hemoglobin < 11 g/dL). Blood lead and hemoglobin concentrations were not correlated in this sample (Spearman rho= 0.17, p>0.1). The scaled scores on the BSID were as follows (Mean ± SD, (range)): cognitive 8.0 ± 2.5 ($2 - 15$), receptive language 8.6 ± 3.3 ($1 - 18$), expressive language 7.9 ± 3.1 ($1 - 18$), fine motor 9.6 ± 3.0 ($3 - 19$), gross motor 9.3 ± 2.7 ($1 - 17$). For the purpose of comparison with other studies, we also calculated Bayley composite scores: cognitive 90.3 ± 12.4, language 90.1 ± 17.4, and motor 96.4 ± 14.6 points. Most children lived with both parents, less than 50% of the mothers were employed, 20.3% of the parents were separated or divorced. Mean maternal IQ score was fairly low, at 81.2 ± 14.2 points. A large number of mothers reported elevated levels of parenting stress (31.7% with PSI T score > 90 points) and depressive symptoms (30.4% with symptoms in the moderate-to-severe range).

B. Unadjusted Associations between Hemoglobin, Lead, and Bayley Scale Scores

When modeled as a continuous variable, higher hemoglobin concentrations were associated with higher cognitive, language, and fine motor scale scores (Table 2, Model 1). Each 1 g/dL increase in hemoglobin was associated with approximately $0.4 - 0.6$-point higher scores on the Bayley scales. BLL was not consistently associated with children's performance on the Bayley (Table 2, Model 2).

When modeled together (Table 2, Model 3), hemoglobin but not BLL was associated with better performance on all Bayley scales (Table 2, Model 3). There were no BLL by hemoglobin interactions. When the above analyses were re-

peated with raw Bayley scores, they yielded similar results (data not shown).

Anemia (Hb <11 g/dL) was associated with lower cognitive ($\beta = -1.6 \pm 0.8$, p<0.05) and expressive language ($\beta = -1.9 \pm 1.0$, p<0.1) scores. Elevated BLLs were not related to children's performance and there were no meaningful interactions between elevated BLLs and anemia. However, children with anemia had raw scores that were 4 – 9 points lower on all Bayley scales (p<0.1 for receptive language and gross motor scales; p<0.05 for cognitive, expressive language, and fine motor scales) than children who did not have anemia, even when anemia was modeled together with elevated BLLs (data not shown). There were no elevated-BLL by anemia interactions on Bayley raw scores.

TABLE I

PARTICIPANT CHARACTERISTICS

Characteristic	N	M ± SD or %	Range
Child characteristics			
Age (months)	69	29.9 ± 8.3	14.4 – 45.6
Sex (female)	69	47.8	
Blood lead (µg/dL)	64	6.0 ± 3.0	2.4 – 15.5
Hemoglobin (g/dL)	69	12.3 ± 1.7	7.7 – 16.1
Child lives with both parents	69	78.3%	
Maternal characteristics			
Age (years)	69	29.0 ± 7.5	16 – 45
Education (years)	69	9.0 ± 3.1	1 – 19
IQ score	69	81.2 ± 14.5	29 – 121
Depressive symptom score >19 points	69	15.7 ± 10.8 30.4%	3 – 52
Total parenting stress T score	69	81.5± 15.5	40 – 99
Employed outside home	69	42.0%	
Family/household characteristics			
Parents separated/divorced	69	20.3%	
Monthly income is variable	69	63.8%	
Socioeconomic status score	69	6.2 ± 2.4	1 – 11
Occupant density	69	1.8 ± 1.1	0.6 – 6
HOME Inventory score	69	9.1 ± 2.0	5 – 14

C. Covariate-adjusted Associations between Hemoglobin, Lead, and Bayley Scale Scores

Each 1 g/dL change in hemoglobin was associated with approximately 0.4-point higher cognitive scores and language scale scores in covariate-adjusted models, although these relationships did not reach statistical significance (p<0.1) (Table 3, Model 1). On the other hand, BLL was not associated with children's performance on Bayley scales (Model 2, Table 3). The association between hemoglobin and expressive language remained statistically significant after BLL was entered into the model (Table 3, Model 3). Two interaction terms between BLL and hemoglobin were significant (Table 3, Model 4, p<0.15) suggesting a differential relationship between increasing hemoglobin, and cognitive and fine motor scores when BLLs were also increasing. The interactions were explored through stratified analyses, modeling BLL as a predictor of scores among children with and without anemia, and modeling hemoglobin among children with low and elevated BLLs. When the interaction was thus explored for cognitive scores, there were no meaningful differences in hemoglobin or BLL estimates in stratified analyses. For the fine motor scale, hemoglobin was positively associated with scores in children with BLLs < 5 µg/dL (0.9 ± 0.4, p=0.042) but not in children with elevated BLLs (-0.2 ± 0.3, p=0.615).

When the above analyses were repeated with raw BSID scores, they yielded similar results (data not shown). Neither anemia nor elevated BLLs were associated with any of the BSID scalar or raw scores in covariate-adjusted models.

IV. CONCLUSIONS

The purpose of this study was to explore the potential interactive effects between hemoglobin and blood lead status in preschool children on their performance on a developmental test. We found that higher hemoglobin concentrations in a sample not generally characterized by poor iron status (anemia) was associated with better developmental test scores. On the other hand, BLL was not a salient predictor of test performance in this sample. We also found some indication that higher hemoglobin concentrations may be associated with better test performance among children who have low (<5 µg/dL) but no higher blood lead concentrations. This finding suggests a possible reduction in the developmental benefit of increasing hemoglobin when lead exposure is present. Nevertheless, we consider this conclusion tentative, in light of the limited sample size, an isolated interaction (seen only on fine motor scores), and lack of association between BLL and developmental scores. Therefore, we were not able to answer whether children with anemia are more susceptible to the effects of elevated BLLs than non-anemic children.

Only a handful of studies assessed interactions between lead exposure and iron status indicators on children's cognition. Ruff et al [19] examined differences in Bayley Scales' mental development index (MDI) in 18-30-month olds who were treated with a chelating agent, iron or both. A decline in BLLs over the 6-month study was associated with better MDI scores of children who were iron-replete but not ID at the study outset. Wasserman and colleagues found no interactions between anemia and BLLs among 24-month old children who had been prospectively assessed from birth [21]. Among Hispanic preschool girls, higher hemoglobin was inversely associated with ratings of aggression and hyperactivity, whereas higher BLLs were associated with increased aggression [36]. Finally, a study of preschool children and their mothers' perceptions of parenting found that higher hemoglobin concentrations were inversely associated with the likelihood of low emotional support scores among children with BLL < 5 µg/dL but not ≥ 5 µg/dL [37].

We found no association between children's BLLs and developmental scores. The evidence concerning the link between lead exposure and young children's neurobehavioral development is somewhat inconsistent, with some studies showing a clear inverse [12], [21], [38] and others showing a null [22], [23], [39] relationship between increasing BLLs and developmental scores. In a longitudinal study of children from Cleveland, Ernhart and colleagues found no consistent associations between BLLs and general development in preschool children [39] or IQ prior to school entry [40]. In young children from Boston, prenatal lead exposure was associated with general development at 24 mo but not later. Furthermore, postnatal BLLs measured up to 24 mo of age were not associated with early child development, whereas later BLLs were [41]. The reason behind these discrepancies is unclear. In Cleveland, a large proportion of the children were exposed to alcohol prenatally [39]. The authors propose that the caretaking environment played an important role in determining children's performance [39], [40]. For the present study, it is possible that in the low-SES sample, social, economic, as well as nutritional factors were more salient determinants of devel

TABLE II

UNADJUSTED ASSOCIATIONS BETWEEN HEMOGLOBIN, BLOOD LEAD CONCENTRATION, AND BAYLEY SCORES IN URUGUAYAN PRESCHOOL CHILDREN

Model	N[1]	Bayley Scale				
		Cognitive	Receptive Language	Expressive Language	Fine Motor	Gross Motor
Model 1 Hemoglobin	66 – 69	0.41 ± 0.17**	0.58 ± 0.23**	0.56 ± 0.22**	0.46 ± 0.22**	0.33 ± 0.20
Model 2 BLL	58 – 61	0.17 ± 0.10*	0.06 ± 0.15	0.09 ± 0.14	0.18 ± 0.12	0.08 ± 0.11
Model 3 Hemoglobin BLL	58 – 61	0.43 ± 0.18** 0.12 ± 0.10	0.62 ± 0.26** -0.02 ± 0.15	0.63 ± 0.24** 0.01 ± 0.13	0.47 ± 0.22** 0.12 ± 0.12	0.36 ± 0.21* 0.04 ± 0.11

[1]Sample size for regression analysis—the larger number refers to the cognitive and language scales, whereas the smaller to the motor scale; [2]Values given as (β ± SE); ***p<0.01, **p<0.05, *p<0.1.

TABLE III

COVARIATE-ADJUSTED ASSOCIATIONS BETWEEN HEMOGLOBIN, BLOOD LEAD CONCENTRATION, AND BAYLEY SCALE SCORES IN URUGUAYAN PRESCHOOL CHILDREN

Model	N[1]	Bayley Scale[2]				
		Cognitive	Receptive Language	Expressive Language	Fine Motor	Gross Motor
Model 1 Hemoglobin	66 – 69	0.39 ± 0.21[3]*	0.45 ± 0.25*	0.42 ± 0.25*	0.24 ± 0.26	0.17 ± 0.24
Model 2 BLL	58 – 61	0.14 ± 0.12	0.12 ± 0.15	0.10 ± 0.14	0.09 ± 0.13	0.02 ± 0.11
Model 3 Hemoglobin BLL	58 – 61	---[4]	0.43 ± 0.29 0.10 ± 0.15	0.45 ± 0.27* 0.08 ± 0.13	---[4]	0.05 ± 0.24 0.02 ± 0.12
Model 4 Hemoglobin BLL Hb x BLL		0.44 ± 0.22* 0.17 ± 0.11 -0.12 ± 0.07*	---[5]	---[5]	0.27 ± 0.26 0.13 ± 0.15 -0.12 ± 0.08	---

[1]Sample size for regression analysis—the larger number refers to the cognitive and language scales, whereas the smaller to the motor scale; [2]Models adjusted for child age, maternal IQ, maternal BDI, maternal PSI score, housing occupant density, HOME Inventory score, and tester; [3]Values given as (β ± SE); [4]The interaction term was significant and reported under Model 4; [5]Hemoglobin by BLL interaction terms for these models were not significant and not reported here. Instead, regressions testing the main effects of hemoglobin and BLL are reported; ***p<0.01, **p<0.05, * p<0.1.

opment in early childhood than lead exposure. This explanation is consistent with a report on Costa Rican children, who experienced cognitive deficits in relation to iron status but not lead exposure in infancy [22]. Additional larger studies are needed to confirm whether or not lead exposure affects early development in this population and if our findings are due to unmeasured factors.

On the other hand, the observed associations of better developmental scores with increasing hemoglobin levels are consistent with previous studies in young children [16], [42]. We do not know whether the anemia in this sample was due to iron deficiency due to lack of other iron status indicators. Nevertheless, there is indication that iron deficiency anemia is a problem in the Uruguayan pediatric population [31] and that low reliance on iron-fortified foods may contribute to this problem [32]. Furthermore, malaria is non-existent in Montevideo and we have no reason to suspect the presence of parasitic infections.

This study had some limitations that may affect the interpretation of the results. First, the study had a small sample size. This study was meant to begin investigating the effects of anemia and elevated BLLs on child development and to identify potentially increased susceptibility to the effects of lead among children whose development is already at risk due to anemia. This question remains unanswered despite years of research on lead exposure and poor iron status as separate insults to children's development [18]. Limited resources precluded us from establishing a larger sample of young children to answer this question, thus additional studies will need to build on our findings.

Another limitation is that we employed translations of instruments originally designed to evaluate child development in U.S. samples. This is not uncommon in low-income settings, where validated and standardized instruments often do not exist [43], [44]. Furthermore, the use of the Bayley Scales allows for some level of comparison with other studies. The Spanish translation of the Bayley employed here has been used with Mexican infants and was pre-tested by our research team for understanding and consistency with Uruguayan terminology. Nevertheless, the Bayley Scales measure general, as opposed to specific aspects of development, and it is recommended that other measures of cognition be employed in future studies.

Our study also had important strengths, including the measurement of socioeconomic status and other factors that influence child development, such as the HOME Inventory, maternal depression and stress, and maternal IQ. The inclusion of maternal factors in particular lends credence to our findings of the independent association between hemoglobin concentrations or anemia and children's scores on the cognitive and language scales.

In conclusion, we found an association between higher hemoglobin levels and higher scores on cognitive and language scales of the Bayley Scales of Infant Development, but no association with BLLs. We also found some suggestion of

a differential association between hemoglobin and fine motor scores in children with low and elevated BLLs, but this finding is tempered by small sample size of the study. We were unable to answer the question of whether anemia increases the susceptibility of young children to the neurotoxic effects of lead. Future studies, with larger samples and additional measures of cognition (as well as behavior) will be needed to shed light on this important issue.

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